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A project on:

Converting an Inkjet Printer into a Bio-printer

Reverse Engineering, Printing & Viability-testing of cell samples

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Introduction - State of the art

Along with the development of 2D/3D printed polymers, there have been efforts for printing living cells or other organic molecules. Multiple scientists around the world have successfully modified commercial thermal and piezoelectric inkjet printers to accomplish this task [3]. The type of printed molecules vary from proteins or other organic molecules to mammalian living cells.

Drop-on-demand bioprinting is commonly used with modified desktop inkjet printers. Modification of desktop printers is both an advantage and disadvantage of this technique. The hardware interfacing with the printhead already exists and has been quality tested by the manufacturer. However, this hardware is designed to print on paper and contains numerous checks to prevent the printer from performing outside its normal specifications. Depending on the bioprinting application, the printer hardware must either be reverse-engineered or modified to bioprint. A printer can be modified to bioprint by using a bioprinting stage on one side of the printer and feeding paper through the other side to bypass the paper checks. Reverse engineering provides control of individual inkjet channels which is extremely beneficial for studying the fundamentals of inkjet bioprinting, especially with regard to channel throughput and clogging. However, reverse engineering a desktop printer may be beyond the capabilities of research groups that do not focus on engineering. Therefore, several groups have used the existing printer hardware with modifications to facilitate bioprinting

One of the major modifications made to desktop inkjet printers is the addition of a Z axis in the paper tray. Since a desktop printer only prints two-dimensional sheets of paper, stacking multiple layers at once requires an additional mechanism to lower the printing area with each successive layer. This is typically performed by adding an electronic elevator to the paper tray that lowers upon receiving a signal from the operator to print another layer. In this way, additional layers can be stacked upon each other to create a three-dimensional construct from two-dimensional layers.

Despite the numerous applications of inkjet bioprinting, there are two major issues that prevent single-cell inkjet bioprinting from reaching its full potential. The first issue is the limited mobility of the printhead if no reverse engineering is performed. In the normal configuration the printhead is limited to less than 250mm on the X axis and the length of the printhead on the Y axis. Thus, only biological structures of limited size can be created with this type of bioprinting system. Second, inkjet cartridges suffer from low throughput. This is mainly due to deposition of salts in the microfluidic channels during the printing process. This often occurs when evaporation of water from the bio-ink drop leaves behind solid salts that block the channel orifice. Once the channel is clogged, it is virtually impossible to restore full functionality to that channel. Furthermore, cellular debris and

other contaminants can clog the microfluidic chambers as well. As a result, inkjet cartridges typically can only print 400,000 cells per cartridge before failure. This throughput is too low to produce large tissue constructs.

It should be noted that older printers tend to produce better results after the modification, as they use ink cartridges with larger diameter nozzles, which do not clog easily and allow to print larger cells. In addition, older printers use mechanical paper feed sensors that are easier to bypass. (Printers with optical sensors can also be "tricked"). Similar attempts have been made using HP DeskJet 500 [4]. Each application of bioprinting requires a separate set of cell types and matrices. Studies of cell viability in thermal inkjet printheads have shown cell survival to be 70–90%, although some groups have determined that cells require a recovery period after bioprinting to restore membrane integrity.

The aim of this project is to convert a typical inkjet printer into a bio/cell printer. The motivation is that bioprinting can significantly contribute to fields of great importance, such as:

- tissue engineering
- regenerative medicine
- direct cell application therapies
- biosensor microfabrication

In addition, a thermal inkjet printer was used for this project, because of the following advantages:

- It is the most common type of printer
- Simple architecture
- Easier to be reverse-engineered
- More suitable for printing cells, better cell-viability than in piezoelectric inkjet printers
- Easy to find older printers, carrying cartridges with larger nozzle's diameter

Description of Inkjet Printer

The most important and sophisticated part of a thermal inkjet printer is the ink cartridge [Figure 1]. Particularly in the case of printing cells, which are very fragile and have a specific volume, the nozzles of the cartridge must be larger than the size of the cells. With that in mind, the selection of the printer for the experimental procedure was significantly affected by the cartridges that this printer carry.

At first an HP DeskJet D4260 printer [Figure 2] was investigated. Since this printer is a relatively new model, the size of the nozzles had to be measured (as it was not provided by the manufacturer), to ensure that is possible to print cell-samples. The same

procedure was carried out for an older printer HP DeskJet 825c [Figure 2] , and finally the printer with the larger nozzle's diameter and drop-volume was selected. The results are introduced in Table 1.

Design Specifications

Printer: HP DeskJet D4260	**Printer: HP DeskJet 825c**
Ink Cartridge specifications: Black: No. 339 (21 ml) Drop: 15 picolitres Nozzles: 672 Nozzle diameter: N/A (not measured) Head Length: 0.5625 in DPI : max 1200 , fast 300, normal 600 ---------- Color: No. 343 (7 ml per color) Drop: 5 picolitres Nozzles: 600 Nozzle diameter ~= 10µm approximately Head Length: 0.5 in DPI: max 1200, fast 300, normal 600 ==========	**Ink Cartridge specifications:** Black: No. 15 (21 ml) Drop: 33 picolitres Nozzles: N/A Nozzle diameter: ~35 µm (measured) Head Length: ~0.5 in DPI : max 600 , normal 300 ---------- Color: No. 17 (7 ml per color) Drop: 9 picolitres Nozzles: N/A Nozzle diameter N/A (not measured) Head Length: N/A DPI: max 1200, normal 600 ========== **SELECTED**

Table 1 - Design specifications of ink-cartridges

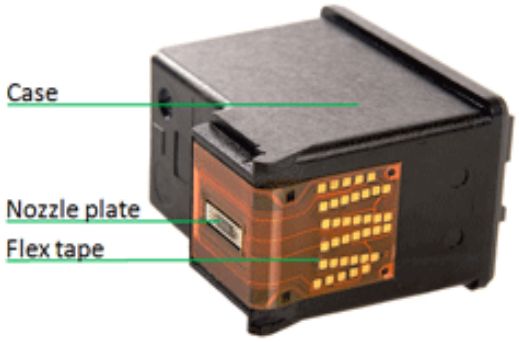

Figure 1 – Main parts of ink cartridge

Figure 2 – Printer selection. Left: HP DeskJet D4260. Right: HP DeskJet 825c

Reverse engineering of inkjet printer

Basic components

We have deconstructed an Officejet J6400 All-in-One printer in order to discover the key mechanisms of the device, and which of those will or could be used for the task we want to accomplish. Both the J6400 and the 825c printer use the same ink cartridges and it is quite possible to have the same mechanisms and control units. What we found is that the mechanisms of the printer are:

1) Ink cartridges/print head holder
2) Paper feeding mechanism
3) Print head dust/air contact protection mechanism
4) Print head cleaning/unclogging mechanism
5) Paper feed sensor (mechanical lever & opto switch) (easy to bypass)
6) Lid status (open/close) sensor (mechanical lever & opto switch) (easy to bypass)
7) Paper and Print head carriage motors
8) Paper motor and print head carriage position feedback and sensors

Protocol for modifying the ink cartridge

Use any long tool with flat edge, for example a straight screwdriver [Figure 5]

1. Remove any tapes/stickers on the cartridge exterior [Figure 3]
2. Remove the metal side panels. Use the tool you have selected as a lever by inserting it from the existing hole [Figure 4, Figure 5]. After the interior of the cartridge will be revealed [Figure 6]. The right panel is needed, don't throw it away.
3. Punch a small hole on the aluminum sheet. There is ink inside so place under running water in a sink until no ink comes from the hole.

4. Remove both the aluminum sheets. They are glued in the border of the plastic cover. Using a sharp tool you must pull it off. Inside there is a spring, which is not needed, and should be disposed.
5. Afterwards, remove the two metallic filters near the printing head (e.g. using tongs).
6. Last step, is removing the soft, light colored plastic inside of the plastic cover. It is quite tricky, but can be done with the same screwdriver. Its edge should be placed between the dark and the light colored plastic. Near the printing head, there is a small button, which is an extension of the light plastic. You must use a knife/saw to cut it from the inside, otherwise the light plastic can't be removed.

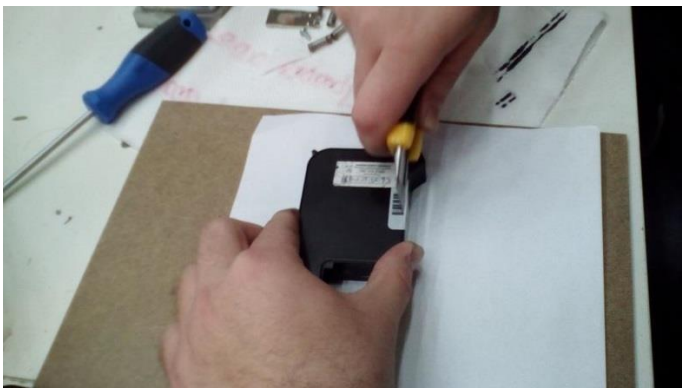

Figure 3 - Removal of any stickers

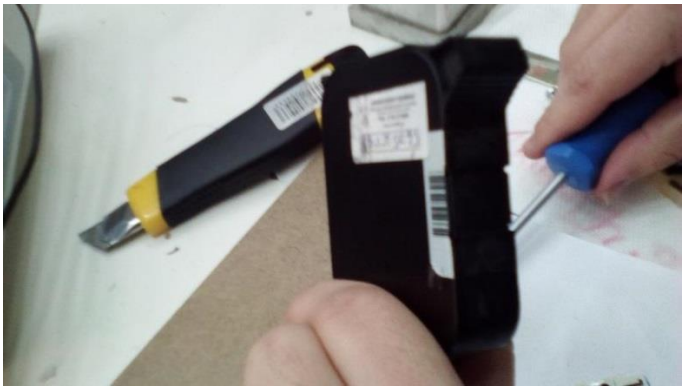

Figure 4 - The entry point for the removal of the side panels

Figure 5 – Usage of flat edge tool

Figure 6 – Ink cartridge after the removal of the panels

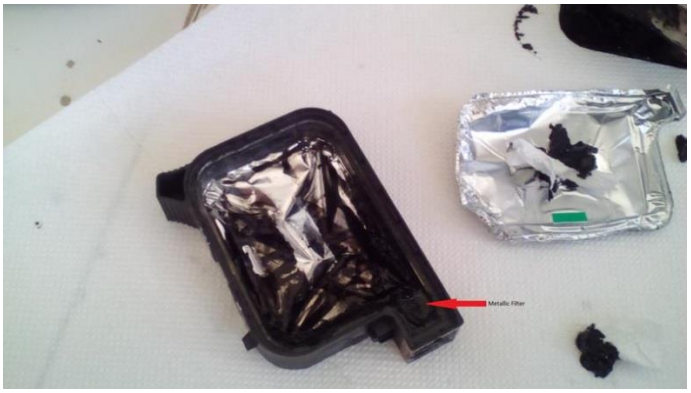

Figure 7 – Ink cartridge after one of the aluminum sheets is removed. The metallic filter can be seen (red arrow). The interior of the cartridge is symmetrical, so the same components are on the other side

Figure 8 - The spring that can be disposed. It lies between the two aluminum sheets

Print head size & structure using microscope for D4260

In order to determine the size of cells that can be "printed" using the Deskjet D4260 printer, it is essential to know the diameter of the nozzles in each of the ink cartridges (blank/color). To do so, we disassembled the print head of an ink cartridge (typically a thin, silver foil with the nozzle holes and ink channels drilled in it) and examined it using two different microscopes.

The nozzle diameter is about 10μm with a 80μm spacing between nozzles [Figure 11]. Only the tri-color ink was disassembled and inspected in detail. The nozzles of the black ink are expected to be slightly bigger because its drop volume is three times larger than the one of the tri-color ink [Table 1], but still it should not exceed 15μm. This allows only the printing of bio-molecules, prokaryotic cells and only small eukaryotic cells with average cell diameter roughly equal to the one of the nozzle.

Ink cartridges contain a sponge which absorbs the main volume of the ink. Between this sponge and the channels that lead the ink to the printing nozzles are placed various filters to keep solid particles from entering and blocking the nozzles. The pore diameter of the main sponge is smaller than 50μm, while that of the filters is way smaller than 10μm. These pores do not allow the ink cartridge to be filled with a cell solution, because most cells cannot pass through and the filters will be quickly blocked. Furthermore, cleaning and sanitizing the cartridge is more difficult if it contains filters. As a result, both the sponge and the filters must be removed, before any other process is initiated.

Another important parameter is the minimum volume of bio-ink required. If the volume of ink is smaller that this quantity, the pressure generated from the inks own weight is not enough to overcome the hydraulic resistance of the channels. As a result, the ink never reaches the nozzles and the printing has very low quality or is not done at all. This parameter can only be measured by experimentation. For this printer its value is 0.5 ml.

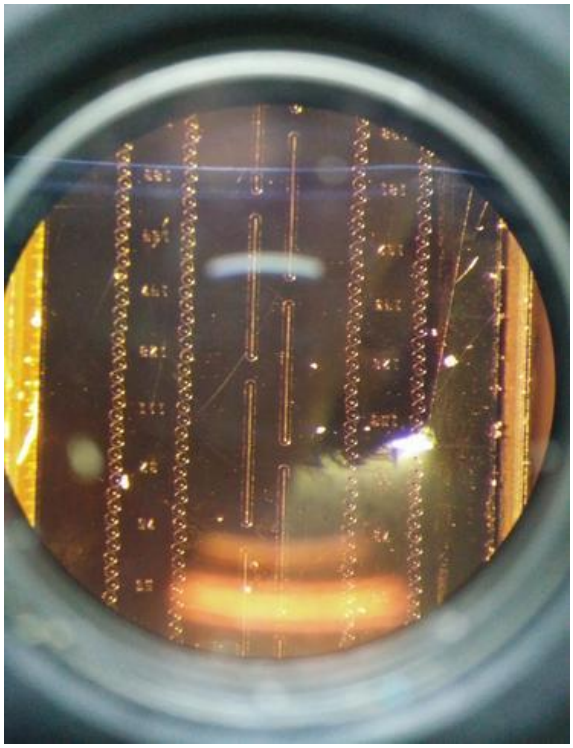

Figure 9 - Black Ink, Nozzles (circles) and Channels (lines)

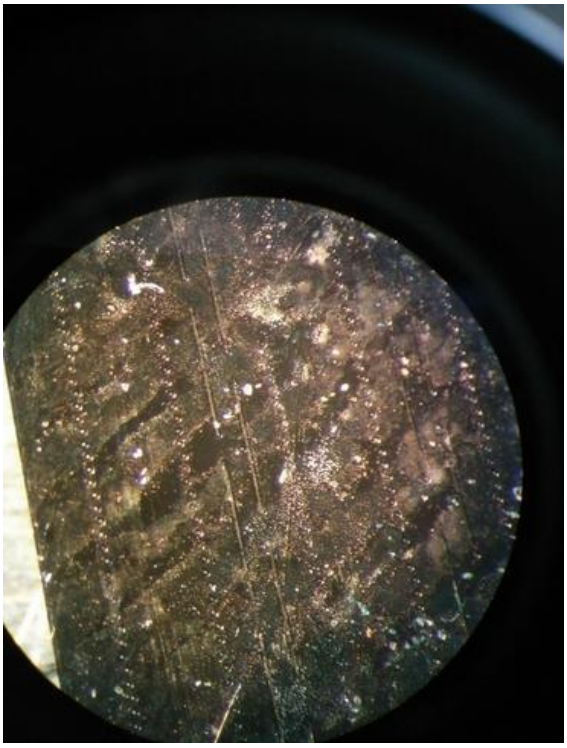

Figure 10 - Tri-Color Ink, Nozzles (circles) and Channels (lines)

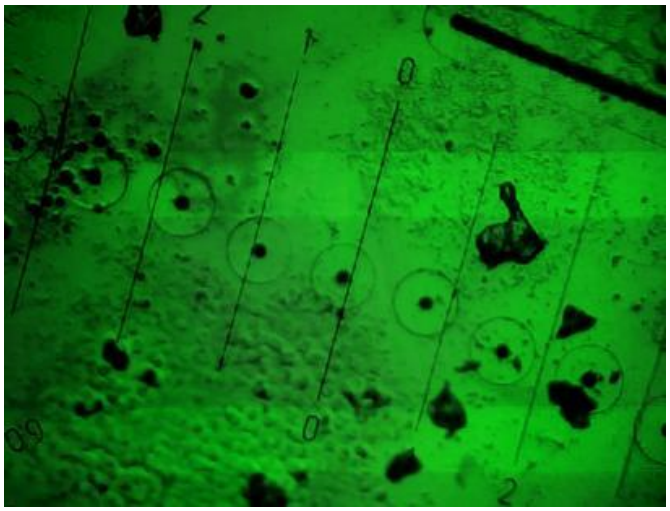

Figure 11 - Tri-Color ink detail, 1-step in scale equals to 0.1mm.Approximate diameter 10µm

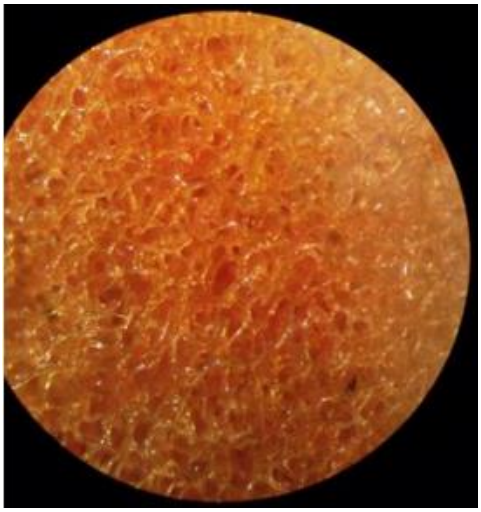

Figure 12 - Main Sponge (pore diameter < 50 µm)

Print head size & structure using microscope for 825c

The measurement was repeated for the black ink of the DeskJet 825c printer, which was also available for experimentation. The nozzle diameter is 35μm, three times larger than that of the D4260, thus allowing the printing of much larger cells. The distance between successive nozzles is about 50μm with an 10μm offset from the straight, central line.

As for the minimum volume of ink of this printer, it was measured with experiments and it is 0.25 ml.

Figure 13 - Black ink, nozzle diameter measurement

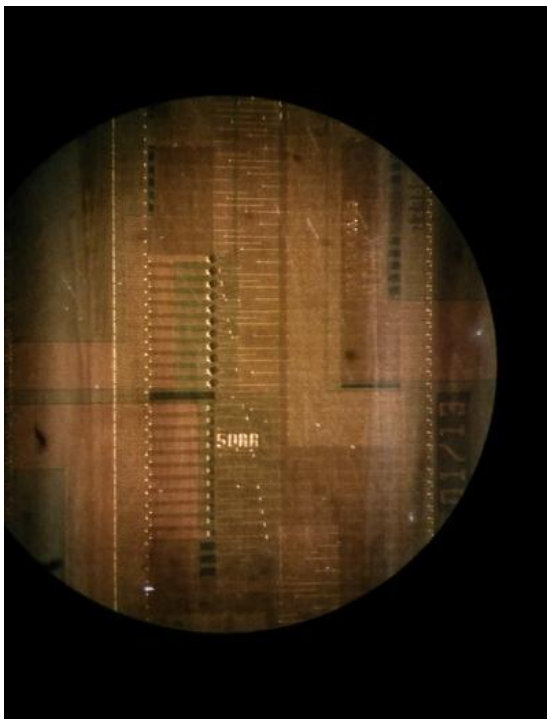

Figure 14 - Black ink, print head detail

Other possible printers – Selected printer

The nozzle diameter of the printer's cartridges disallows most eukaryotic cells to be printed without serious stress. As a result, older printers that use cartridges with larger nozzles had to be found. One of the most suitable option is the HP 26 Black Ink Cartridge [2.1] with a drop volume of 140-150 picoliters (>10 times larger than the HP 339 Ink) and a nozzle diameter of about 50μm. Unfortunately, most printers that use this cartridge are not sold today. Additionally, it is unclear if these printers will still work during the lifetime of the project and whether spare parts and cartridges will be marketable the following years.

There are also some custom made print heads, usually controlled by an arduino device. They offer very low DPI values and accordingly large nozzle diameters. One of these is the InkShield with 96x96 DPI and nozzle diameter equal to 85μm, features that make it ideal for the purpose. The price of an InkShield DIY Kit is about 60$ [2.2].

To summarize, there are some good but limited options available in the market. Nevertheless, in this project an already owned printer will be used and an upper limit will be set on the diameter of cells that can be printed. Both printers, D4260 and 825c, were reversed engineered and reached a level, where they could be manually operated to print cells or biomolecules. It was important, though, to choose only one of them for further experiments, automatize its printing process and modify it, until it reaches the desired design level. The main factor that affected this choice was the nozzle diameter and the range of cells that can be printed. Thus, the DeskJet 825c is considered, as previously mentioned, a superior choice, with the following advantages:

- Three times larger nozzle diameter
- Easier to use and reverse-engineer ink cartridges
- Smaller minimum volume of bio-ink required
- Simple design
- Larger space available for items that will be used as printing surfaces

Sensors and systems to be tricked/used

In the following figures [Figure 15-18] the main sensors that need to be tricked are shown.

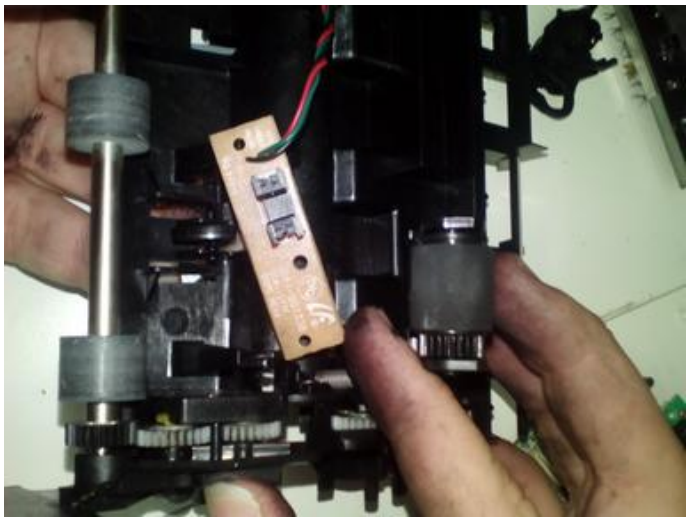

Figure 15 - Paper feed sensor opto switch

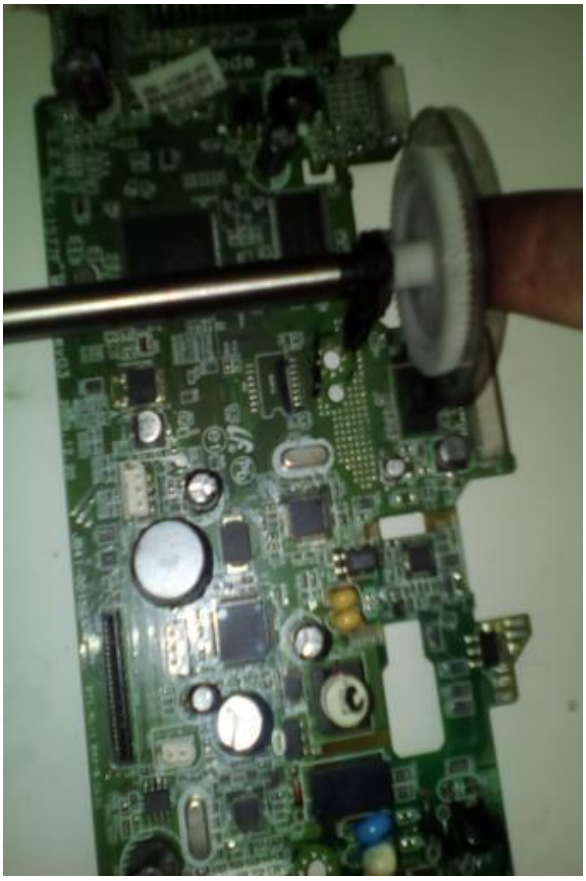

Figure 16 - Rotational photosensor paired with the paper axis

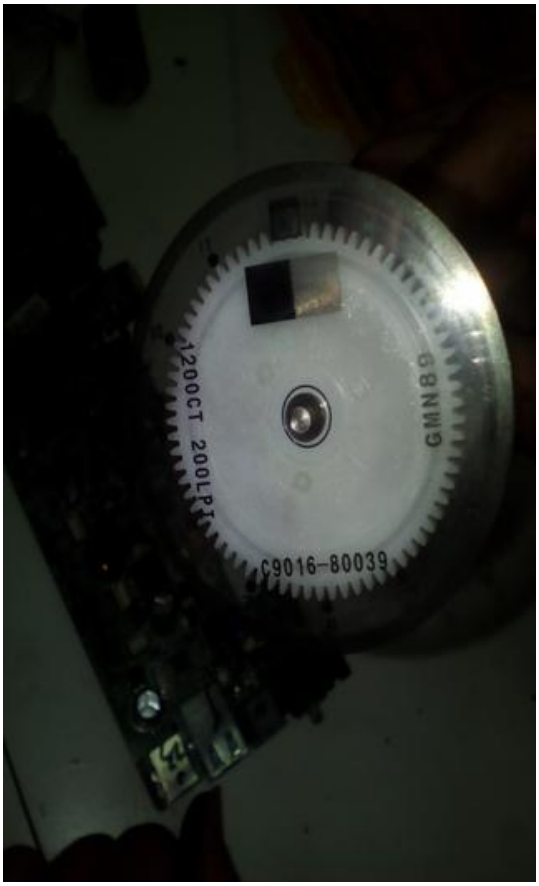

Figure 17 - Encoder for the rotational photosensor

Determining the sequence of functions during the printing

In order to modify the printer, regarding the printing sequence and the way the printer uses its sensors, it was necessary to record the printer's actions while printing normal ink on normal paper. It was easy to trick the lid sensor, but without removing any of the functional components the paper feed sensor is quite tricky to overpass.

The printer body is to be kept. The main structure of the printer will be used, but excess parts will be removed, as the head cleaning tray, the paper transport tray and part of the axis that transfers the paper while printing. Although the main paper feed motor is not directly needed, it will be kept because it helps tricking the feed sensor.

Concerning that, an Arduino micro-controller will be used. The controller will get signal from the main motor. When the paper sensor is enabled, the motor change rotation for an instant. In correlation to the fact that the motor is DC powered, that change in the voltage [Figure 18] will be used to signal the controller. In its turn, the controller turns on an external infrared led emitter (the appropriate voltage was measured, Figure 19) which

signals the sensor instead of his own (blocked) emitter. In that way, we use the original circuit of the printer without any internal changes, which guarantees its function.

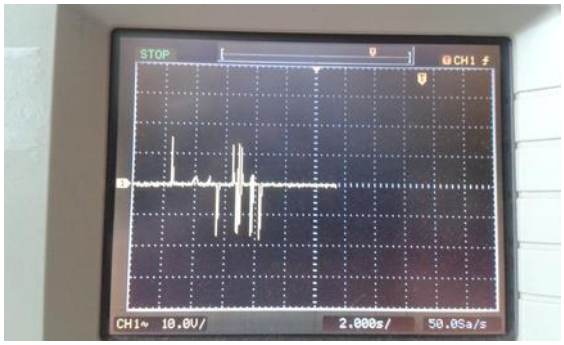

Figure 18 - Motor voltage (oscillograph monitor)

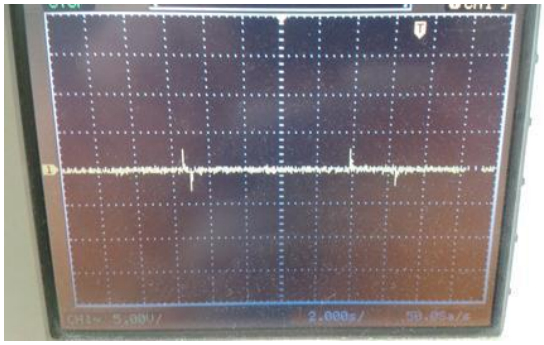

Figure 19 - Sensor emitter voltage (Oscillograph monitor)

The full cleaning & printing protocols can be found at Appendix A

The circuitry and the arduino code can be found at Appendix B

Figures of the final printer-design can be found at Appendix C

Experimental procedures and results

The following experiments are considered important to determine the survivability of cells through the whole procedure (bio-ink fabrication, storage in ink cartridge, printing):

Test type	Cell Type	Results
1) Test viability of cell sample	Yeast	Success
2) Test viability after printing	Yeast	Success
3) Test viability after adding printing enhancer (e.g. glycerol)	Yeast	Not needed**
4) Test viability of cell sample	Other*	Success
5) Test viability after remaining for 20 minutes in ink cartridge	Other	Success
6) Test viability after printing	Other	Success
7) Find bio-compatible ways to regulate bio-ink's viscosity	All	Not needed

Table 2 - Survivability experiments

* Other cell types must be declared (fiborblasts, chondrocytes, cartilage tissue)

** "Not needed" indicates that the printing is successful without the need to regulate the bio-ink's viscosity. The viscosity regulation presupposes a reliable way to measure it as well as a reliable method to calculate the cell's density in the ink. Both measurements could be done with great difficultly or not at all in a cell solution.

Cell type	Yeast	Fibroblast
Live cells before printing	119	25
Dead cells before printing	28	13
Viability before printing	80%	65%
Live cells after printing	106	17
Dead cells after printing	77	42
Viability after printing	60%	30%
Total Survivability (through printing process)	**75%**	**50%**

Table 3 – Experimental results

In the beginning, yeast samples were used in the experiment, because yeast is a smaller dimension cell, so the printing would be easier to happen. The process was successful [Figure 20]

Figure 20 – Yeast printing-experiment on glass

The following images display the results of the experiments 5,6. Two tests were carried out with the same cells (fibroblasts). On the left are the control samples while on the right are the printed cells. Both of the samples are colored with trypan blue in order for the survivability of the cells to be measured.

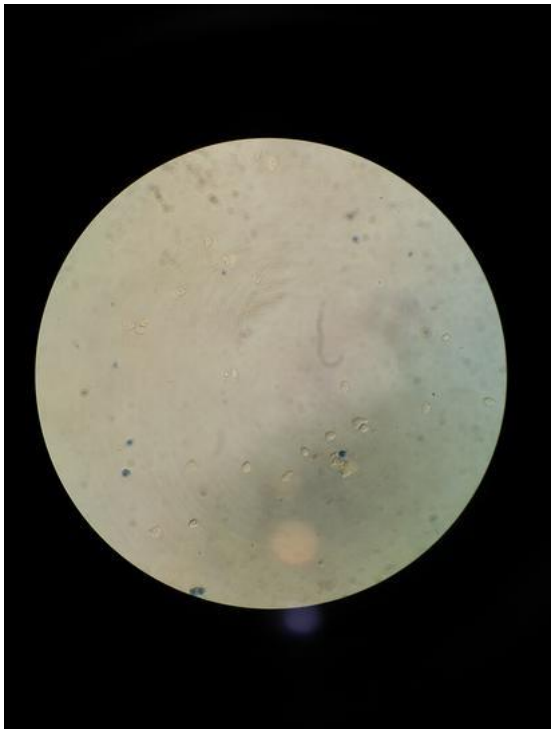

Figure 21 - Fibroblast cells before printing 1 (Control sample 1)

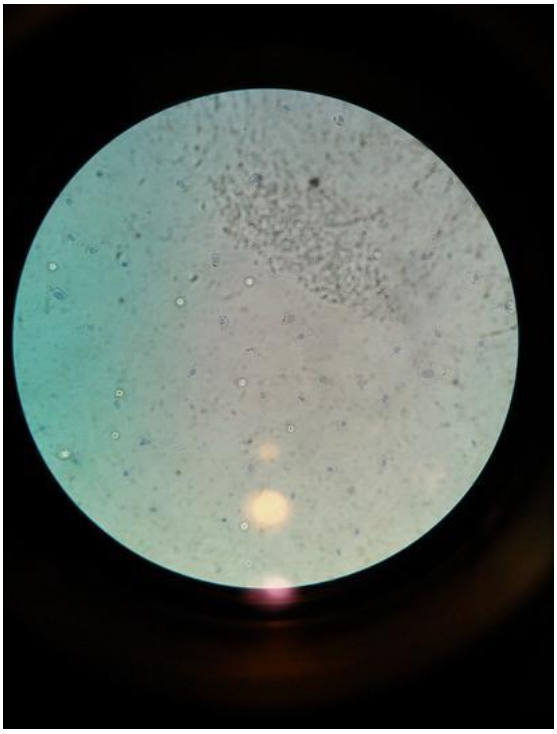

Figure 22 - Fibroblast cells after printing 1 (Test sample 1)

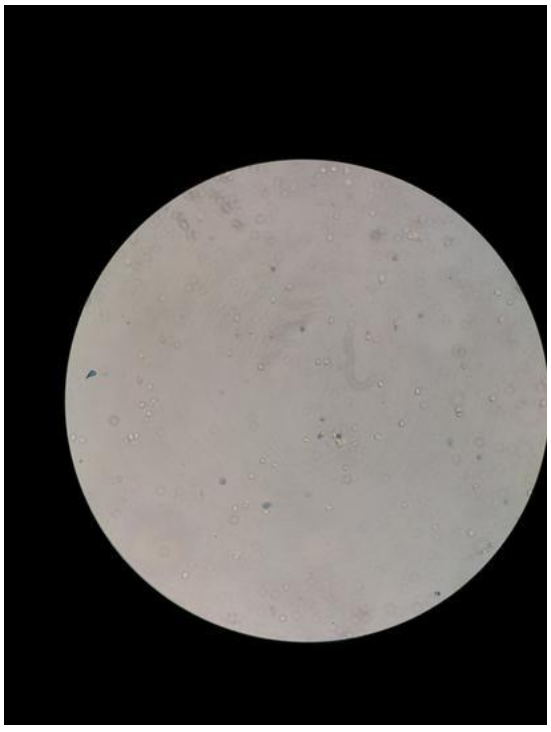

Figure 23 - Fibroblast cells before printing 2 (Control sample 2)

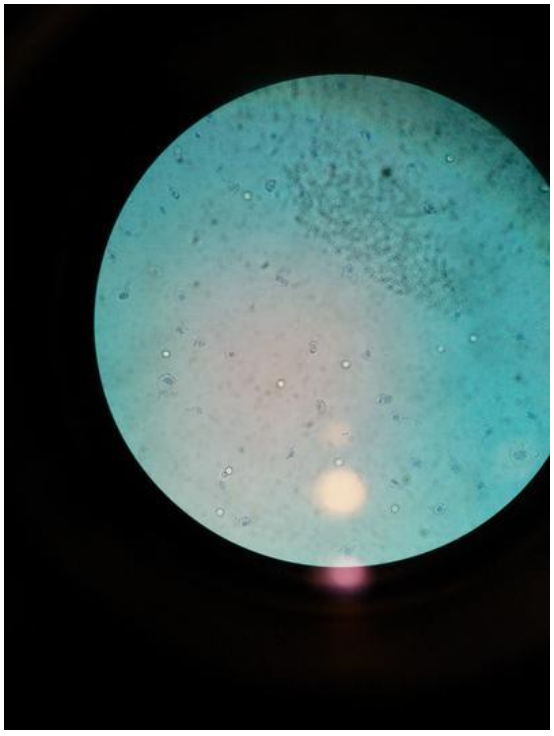

Figure 24 - Fibroblast cells after printing 2 (Test sample 2)

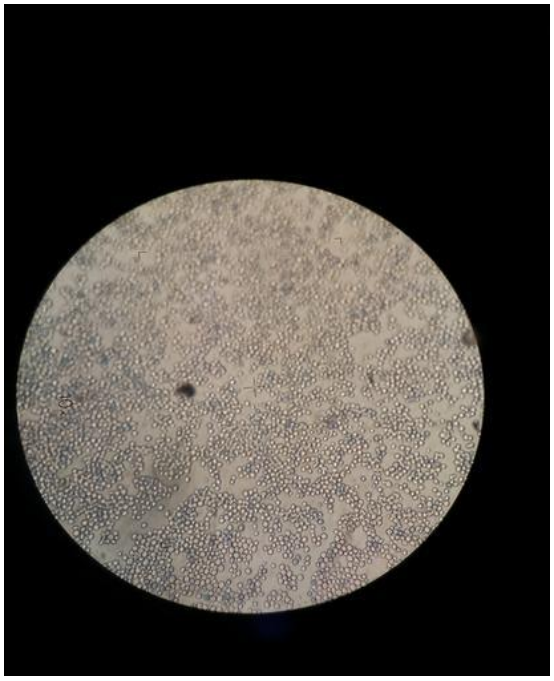

Figure 25 - Yeast before printing

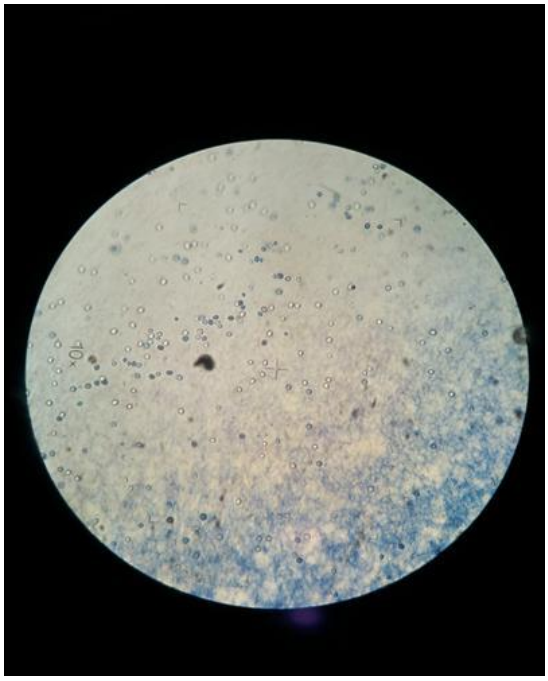

Figure 26 - Yeast after printing

Future prospects

This project surely has the potential to constitute the base for future works concerning bio-printing with cheap and easy to find equipment. There are still a number of issues that has not been investigated in the current project. Such issues that can be solved in the near future are:

- Construction of the appropriate cover in order to avoid direct contact with specific parts of the printer (mostly for safety reasons)
- Manually adjustable printing-stage (so as to print on different surfaces)

Some more sophisticated future directions concerning this project are:

- Construction of an automatically adjustable moving stage in y-axis (paper-feed axis)
- Construction of an automatically adjustable moving stage in z-axis. Ability to print 3D bio-components
- Automation of the pre-printing procedure (e.g. clean cartridge, add cell-sample to cartridge, adjust petri-dish to stage)
- Automation of the post-printing procedure (e.g. transfer petri-dish to microscope, examine cell-sample)

References

Research Papers

1.Human Cartilage Tissue Fabrication Using Three-dimensional Inkjet Printing Technology
http://www.jove.com/video/51294/human-cartilage-tissue-fabrication-using-three-dimensional-inkjet
2. Inkjet printing of viable mammalian cells:
http://www.sciencedirect.com/science/article/pii/S0142961204003357
3. Printing technologies for biomolecule and cell-based applications:
http://www.sciencedirect.com/science/article/pii/S0378517315001337?np=y
4.Creating Transient Cell Membrane Pores Using a Standard Inkjet Printer
http://www.jove.com/video/3681/creating-transient-cell-membrane-pores-using-a-standard-inkjet-printer
5. Inkjet printing for high-throughput cell patterning:
http://www.sciencedirect.com/science/article/pii/S0142961203010093
6.**Drop-on demand inkjet bioprinting: a primer**
http://www.worldscientific.com/doi/abs/10.1142/s1568558611000258

Web sites
2.1
http://store.hp.com/UKStore/Merch/Product.aspx?id=51626AE&opt=&sel=SUP
2.2
http://nerdcreationlab.com/Store/

Appendix A: Experimental protocols

Protocol for cleaning the printer:

1. Leave the printer body under UV light for at least 20min
2. Spray 70% ethanol on any surface, and especialy the ink holder

Protocol for cleaning ink cartridge:

1. The ink cartridge should be cleaned before and after use.
2. Fully submerge the cartridge in a beaker full of de-ionized water, and sonicate for 15 minutes before and after printing.
3. After sonication, remove the cartridge from the water, and shake out excess water.
4. Spray 70% ethanol into the cartridge to create a more aseptic environment. (!Note: Ensure that the ethanol has dried before adding bioink-cells)

Protocol for printing samples:

1. Clean the printer body and the ink cartridge
2. Prepare the sample pattern in a text editor (e.g. Microsoft Word-Windows, LibreOffice-Ubuntu)
3. Connect the printer to a computer and the power supply. Connect the power supply of the Arduino micro-controller!
4. Set the printer power on and wait for the machine preparations (~30 sec). The end will be defined by the positioning of the ink carriage on the right of the body and the halting of movement. Upper green light should be constantly lit
5. Set the appropriate printing surface

   Appropriate dimensions:
   Length: <10 mm (strictly!)
   Width < 210 mm (with the value depending on the border option inside the text editing software)
   Thickness: =<500 mm

6. Supply the ink cartridge with the cell solution and place it in the ink carriage
7. Send print command from the computer
8. Wait until the ink carriage returns at its original position
9. Remove the printing surface

In order to repeat the process re-cleaning of the printer body (especially when printing different cell types) is highly advised and re-cleaning of the ink cartridge is mandatory

Printer warnings/errors

The HP DeskJet 825c has three buttons (from up to bottom, Figure 2):

- Power button (turns green when the printer is powered on)
- Resume button (middle button-turns orange when there is a paper jam)
- Cartridge status button (turns orange when the printer does not identify the cartridge)

When the Resume button turns on, turn off the printer and turn it on again.

If the problem insists then:

- Turn off the printer
- In the arduino code comment lines 31-47 and uncomment line 30
- Upload the program to arduino (with arduino IDE)
- Open the printer
- When the power button turns green and the cartridges are to the 'home' position change the arduino code to its original form and upload the program again (with arduino IDE)
- Then the printer is ready to function

When the Cartridge status button turns on:

- Remove the paper that blocks the lid sensor (to virtually open the printer's lid)
- Wait for the cartridges to move to the center-position
- Remove the cartridge, clean the flex tape gently, and place it back in the printer
- Block the lid sensor with the paper (to virtually close the printer's lid)
- Wait for the cartridges to move to the 'home' position
- The Cartridge status button should have turned off and the printer should be ready to print

If the problem insists, it means that the cartridge is identified as empty from the printer. The cartridge can be reset or replaced. Then the printer is ready to function.

Appendix B: Arduino Code/Circuitry

Arduino code

```
/*----------------------------------------
------    BIOPRINTER PAPER-HACK      ------
----------------------------------------*/
//This program works for
//Printer: HP DeskJet 825c
//Single-page printing
//(For printing more pages you have to change the Adjustable Time
(Line 43 [milisec])

//Global variables definitions
int motorPin=0;
int sensorPin=7;
int motorValue;
int thres=2.5;
float motorVoltage;

//Setup function
void setup(){
  pinMode(motorPin,INPUT);
  pinMode(sensorPin,OUTPUT);
  Serial.begin(9600);    //Initialize serial communication at
9600 bits per second
  Serial.println("Initialization complete\n");
  Serial.println("Paper sensor opened");
}

//Main loop
void loop(){
  motorValue=analogRead(motorPin);
  motorVoltage = motorValue * (5.0 / 1023.0);
  Serial.println(motorVoltage);
//     digitalWrite(sensorPin,HIGH);         //In case of error
to restart due to paper-jam uncomment this line and comment lines
31-47
  if (motorVoltage>thres){
     Serial.println("Motor starts moving");
     Serial.print("Threshold exceeded with Voltage : ");
     Serial.println(motorVoltage);
     delay(2000);                           //Delay for 2 sec

     digitalWrite(sensorPin,LOW);           //Paper-sensor
"Closed"
     Serial.println("Paper sensor closed");
     delay(2000);                           //Delay for 2 sec

     digitalWrite(sensorPin,HIGH);          //Paper-sensor
"Opened"
```

```
     Serial.println("Paper sensor opened");
     delay(15000);                              //Delay for 15 sec
(for printing to end) - Adjustable Time
  }
  else{
     digitalWrite(sensorPin,HIGH);              //Keep paper-sensor
"Opened"
  }
  delay(1);                                     //Delay for 1   ms
}
```

Arduino circuitry

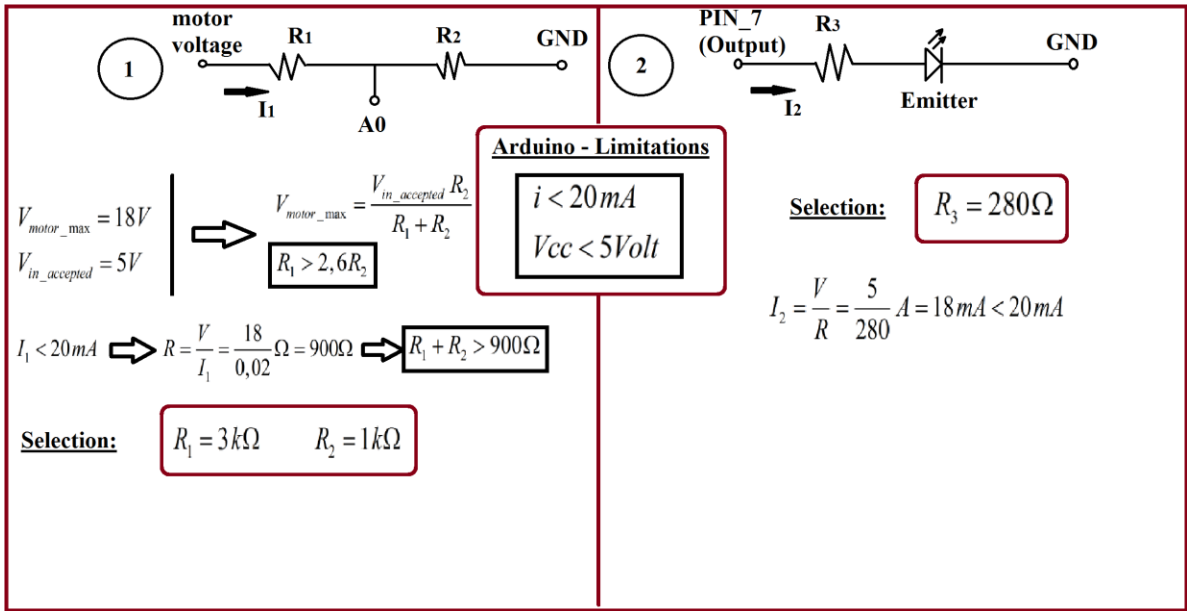

Figure 27 – Arduino Circuitry

Appendix C: Printer-design

In the following figures [Figures 28-32] the final design of the printer is shown.

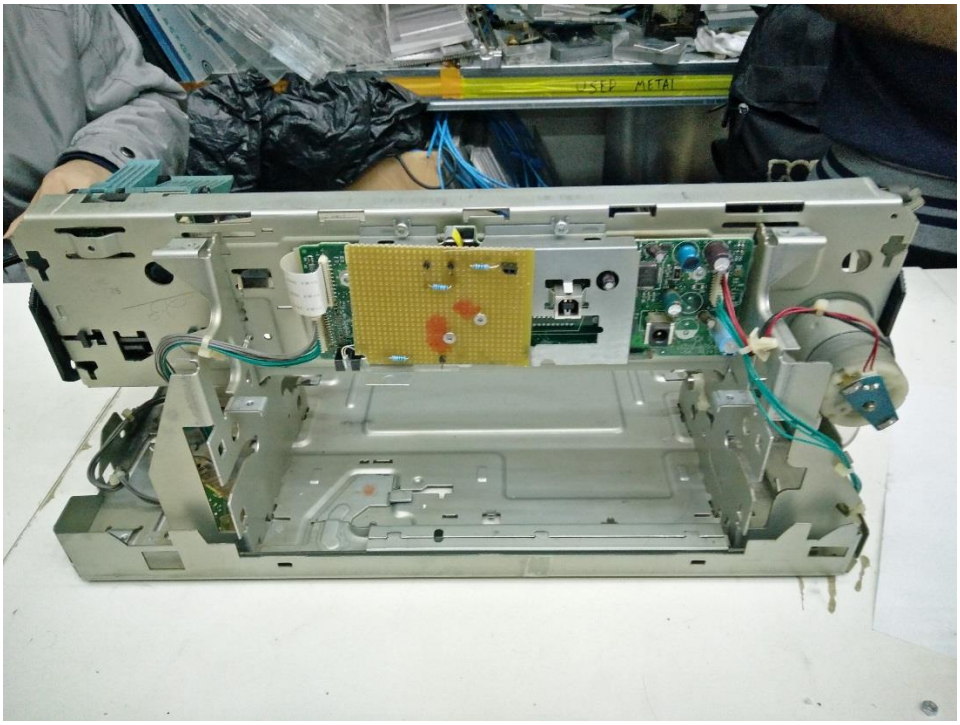

Figure 28 – Back view of converted inkjet printer

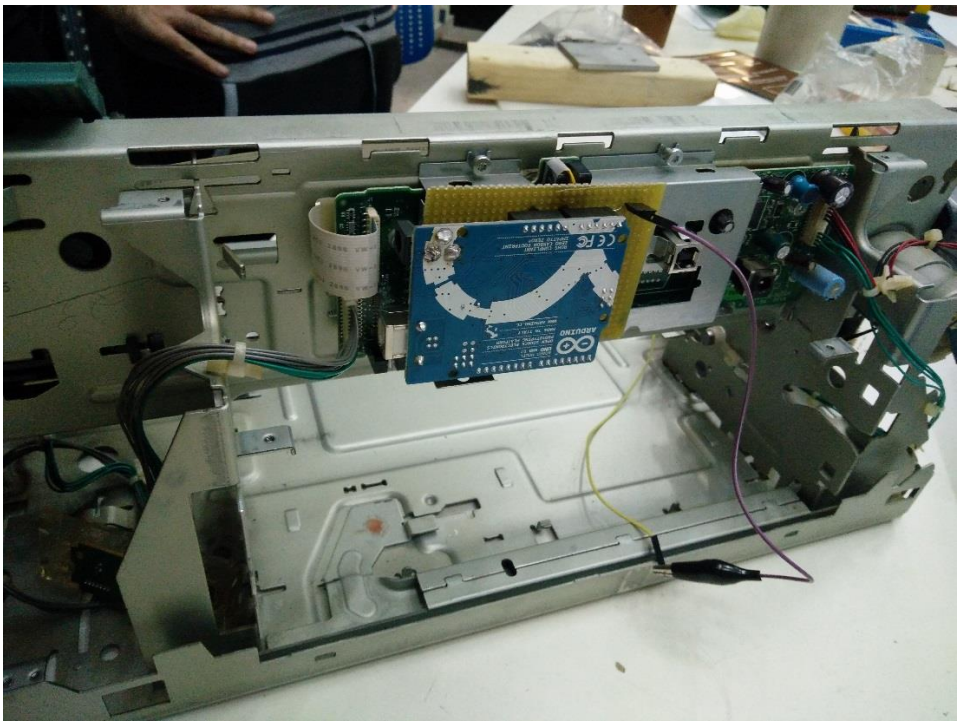

Figure 29 - *Back view of converted inkjet printer / Connection with arduino*

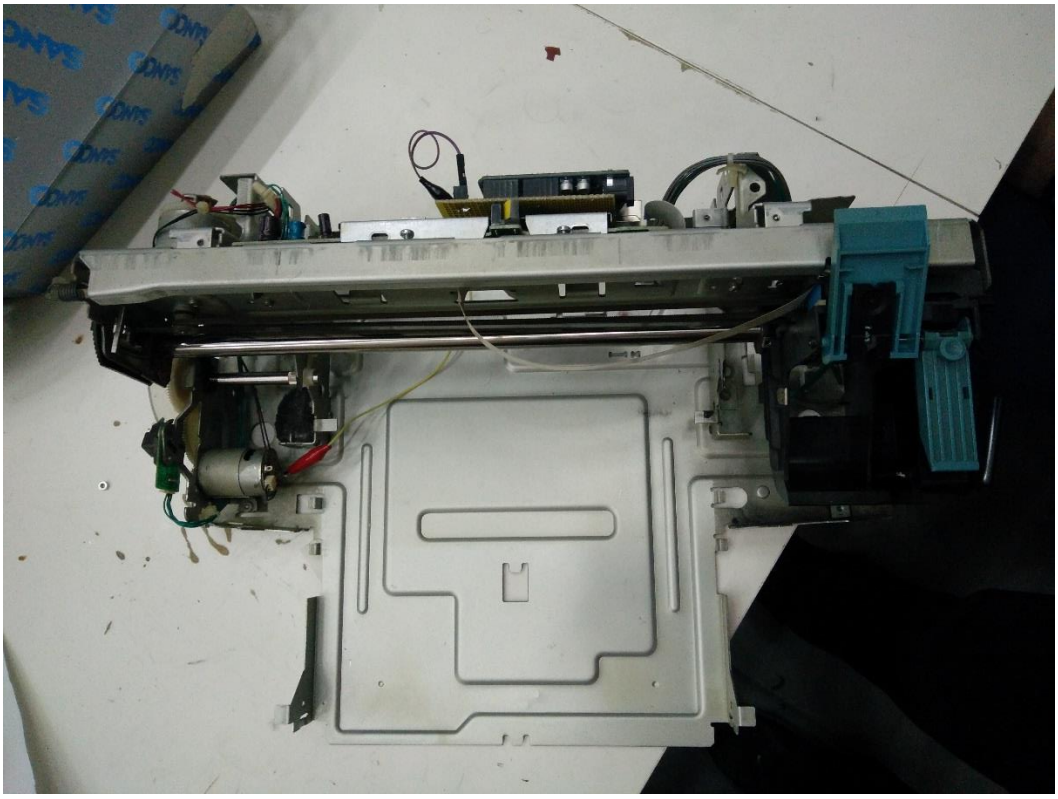

Figure 30 – Top view of converted inkjet printer / Connection with motor

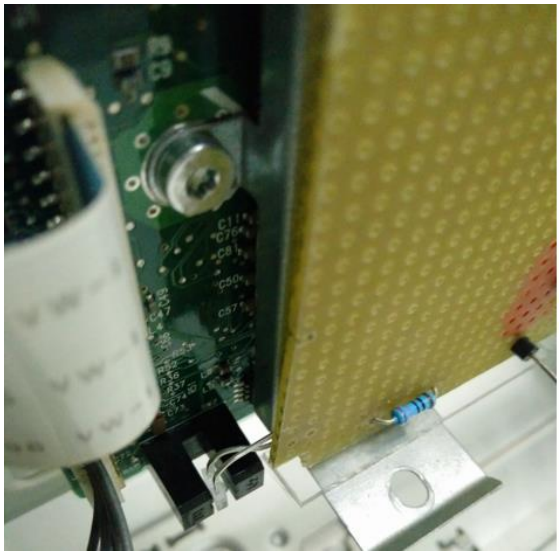

Figure 31 –Position of the emitter of the additional circuitry / Bypass of the printer's original emitter

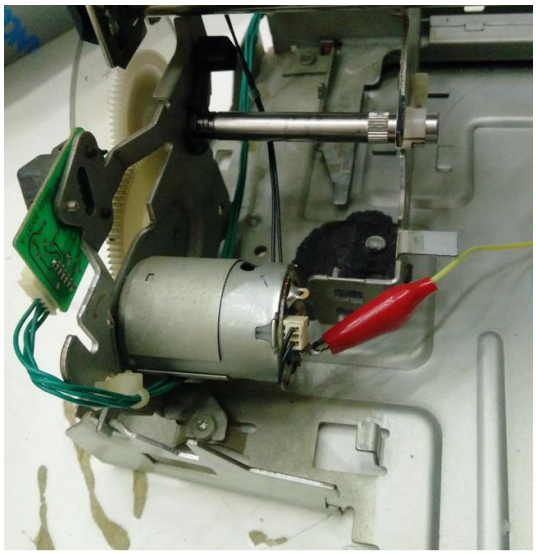

Figure 32 –Detailed view of the connection with motor